Subliminal Stimulation and Inhibition of Visual Processing

Dissertation

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Abstract

Walking along a street on a moonlit night, we can perceive visual events at an enormous range of intensities – from the blinding light of a passing motorcycle to faint shadows filling the night with their different shades of darkness. Does the visual system react similarly to all of these events? In this thesis, I investigated how the visual system reacts to stimuli that are so low in their intensity that they are not perceived.

It has been shown that subliminal low-intensity somatosensory stimuli lead to a cortical deactivation or inhibition in somatosensory areas. This deactivation was interpreted as a focal inhibition mechanism that usually protects the cortex against activation by noise. The proposition was supported by a behavioural experiment, showing an increased sensitivity threshold for peri-liminal stimuli during subliminal stimulation.

I investigated whether such inhibitory mechanisms can be found within the visual system as well. In Study I, I investigated the perception of visual peri-liminal target-stimuli under three different conditions. Compared to target-stimuli presented alone, the threshold for target-stimuli significantly increased when they were presented during subliminal stimulation on the same side as the target-stimulus. I hypothesized that the underlying mechanism of this effect is mediated through intracortical inhibition. Concurrently, studies in macaque senescent neurons suggest a degradation of intracortical inhibition with age. In Study II I therefore investigated differences of inhibitory responses in a group of elderly subjects and compared the results to the young participants in Study I. Results indicated that two out of five elderly subjects showed the inhibitory effect of subliminal stimulation compared to six out of seven in the group of young subjects. The difference between the two groups was not statistically significant. Future studies will require a larger sample size in order to clarify this issue. Additionally, other explanations beyond sample size are discussed. In Study III, using electroencephalography, I show that subliminal stimulation leads to an alpha-band power increase, whereas supraliminal stimulation leads to a lower frequency increase as well as alpha-band power decrease. The specific neural signature in response to subliminal stimulation indicates neural processing of the stimulus that leads to a down-regulation of areas involved in stimulus processing. This mechanism could serve a suppression of input noise that has been described in the somatosensory system and may lead to decreased detection of peri-liminal target-stimuli during subliminal stimulation.

Zusammenfassung

Bei einem nächtlichen Spaziergang im Mondlicht, nehmen wir Ereignisse verschiedenster Intensität wahr. Vom blendenden Licht eines vorbeifahrenden Motorrads bis hin zu undeutlichen Schatten. Verarbeiten wir diese Ereignisse alle gleich? In dieser Arbeit untersuchte ich, wie das visuelle System auf Stimuli reagiert, die so niedrig in ihrer Intensität sind, dass wir sie nicht wahrnehmen. Frühere Studien haben gezeigt, dass das somatosensorische System auf subliminale Stimuli mit einer kortikalen Deaktivierung oder Inhibition reagiert. Diese wurde als Hemmungsmechanismus interpretiert, welcher kortikales Rauschen unterdrückt. Unterstützt wurde diese Aussage durch ein Verhaltensexperiment, in dem der Schwellwert für somatosensorische Stimuli bei subliminaler Stimulation erhöht war.

Gibt es im visuellen System äquivalente Hemmungsmechanismen? In Studie I dieser Arbeit untersuchte ich die Wahrnehmung schwellnaher visueller Zielreize: Im Vergleich zu Zielreizen, die alleine präsentiert wurden, war die Wahrnehmung bei gleichzeitiger subliminaler Stimulation im selben Hemifeld verschlechtert. Auf der Grundlage verschiedener Studien vermuten wir, dass der Effekt durch inhibitorische Interneurone hervorgerufen wird. Gleichzeitig zeigten Nervenzell-Studien die Degeneration intrakortikaler Inhibition mit dem fortschreitendem Alter von Affen. In Studie II untersuchte ich Unterschiede inhibitorischer Mechanismen einer kleinen Gruppe von älteren Probanden und verglich diese mit den jüngeren Probanden aus Studie I. Die Resultate weisen darauf hin, dass zwei der fünf älteren Probanden einen inhibitorischen Effekt zeigen, gegenüber sechs von sieben der jüngeren Probanden. Dieser Unterschied wurde statistisch nicht signifikant. Verschiedene Erklärungen hierfür werden diskutiert. In Studie III, einer elektrophysiologischen Studie, führt subliminale Stimulation zu einer Verstärkung des Alpha-Rhythmus. Supraliminale Stimulation führt zu einer Verstärkung niedriger Frequenzen und einer Abschwächung des Alpha-Rhythmus. Die spezifische neuronale Signatur aufgrund subliminaler Stimulation deutet darauf hin, dass die neuronale Verarbeitung des Stimulus zu einer Verringerung der Aktivität in involvierten Arealen führt. Ein Rauschunterdrückungs-Mechanismus wurde im somatosensorischen System beschrieben und könnte für die verringerte Wahrnehmung der schwellnahen Zielreize bei subliminaler Stimulation verantwortlich sein.

List of Papers

This thesis is based on the following original papers:

Study I

Isabelle Bareither, Arno Villringer, Niko A.Busch. Decreased visual detection during subliminal stimulation. Journal of Vision. 2014. 14(12): 20, 1-9.

Study II

Isabelle Bareither, Arno Villringer, Niko A.Busch. Subliminal stimulation and inhibition of visual processing: investigating differences between young and elderly. (Pilot-Study)

Study III

Isabelle Bareither, Maximilien Chaumon, Fosco Bernasconi, Arno Villringer, Niko A. Busch. Invisible visual stimuli elicit increases in alpha-band power.
Journal of Neurophysiology. 2014. 112: 1082 – 1090.

1. Introduction

Walking along a street on a moonlit night, we can perceive visual events at an enormous range of intensities –from the blinding light of a passing motorcycle to the faint glimpse of its backlight disappearing in the night. Faint shadows around us fill the night with their different shades of darkness. Some of them we perceive, some of them are too faint to become visible.

How does the visual system react to all of these intensities? Does it respond similarly to the blinding light and the invisible shadow? In this thesis, I investigated in three studies how the visual system reacts to subliminal stimuli – stimuli that are so low in their intensity that they cannot be consciously distinguished from the background. In a first study, I investigated behavioural responses to peri-liminal stimuli when presented during subliminal stimulation. In a second study, I tested whether these responses change with increasing age in a group of elderly participants. In a third study, I investigated neural responses to high-intensity supraliminal stimuli as well as low-intensity subliminal stimuli.

2. Theoretical Background

2.1. Subliminal Perception

The scientific study of human non-conscious processes is one of the most interesting and also one of the most controversial. As early as the 19th century, it was suggested, that participants perceive more than they would be consciously aware of (Sidis, 1898): a number of letters was presented from such a distance that participants indicated they could only guess the answer. Surprisingly, they performed above chance. Enthusiasm and critique followed these studies. Anthony Marcel (Marcel, 1983) was one of the first researchers, who implemented unconscious perception into existing cognitive theories. He could show that the very well established phenomenon of priming can also be found if the prime was made invisible by a masking stimulus. These experiments sparked a long-lasting scientific interest in the study of unconscious processes, as well as continuous discussion about methodological considerations. Until today, questions surrounding subliminal processing concern on one side the definition of a threshold for non-conscious perception as well as adequate measures to test this threshold and on the other side the definition of the term 'subliminal'.

2.2. The threshold for subliminal perception

Considering the detection of a faint shadow on a moonlit night – where is the threshold for perception, and how can it be measured adequately? According to psychophysical theories, an *absolute* threshold is the intensity at which an observer is able to detect a repeatedly presented stimulus a certain number of times (e.g. 50% chance level in yes/no paradigms). However, as has been pointed out within the framework of signal-detection theory (Green & Swets, 1966), in uncertain circumstances (e.g. around chance level), an observer will never answer purely objectively to the question whether he/she has seen the stimulus. An observer has to detect the signal (e.g. the shadow) on a noisy background full of visual distractors (e.g. trees, moonlight). Additionally, the likelihood of detecting a certain shadow can change with slips in attention or increasing tiredness. Importantly, detection-rates can also change with the bias of an observer towards a certain decision. For example, if the observer is expecting someone, detection of shadows will likely be higher. Therefore, if we would present the same near-threshold shadow to an observer on the street several times, he might not detect it on one trial, but very well detect it on another. According to signal-detection theory, the threshold for perception can be calculated by considering Hits (stimulus present

and detected), False Alarms (stimulus absent, but detected), Miss (stimulus present, but missed) and Correct Rejection (stimulus absent and not detected). By considering the bias of the observer (False Alarms) and sensitivity, numerical estimates of a sensitivity-index (such as d' or D-prime) can be derived.

2.3. The definition of subliminal processing

Considering a shadow that is below the absolute threshold, is it processed 'subliminally'? Within the *global neuronal workspace* (GNW) model (Dehaene, Changeux, Naccache, Sackur, & Sergent, 2006), two processes below the threshold of perception are being proposed: subliminal processing and pre-conscious (or potentially conscious) processing. The concept of pre-consciousness refers to a neuronal activation that potentially entails enough activity for conscious access, but is not accessed because of a lack of attention. This pre-conscious processing could occur in attentional-blink paradigms or change-blindness paradigms. On the other hand, subliminal stimulation refers to neuronal activation that does not entail enough activity for conscious access, even though it is attended to. Dehaene, et al. (2006) refers to stimuli that are presented at threshold, attended, but nevertheless not perceived.

In this thesis, the presence of the subliminal stimulus was imperceptible from the noise-background even though participants were asked to covertly pay attention. Therefore, the imperceptible stimuli can be seen as subliminal in the sense of the GNW Model.

2.4. The nature of the subliminal stimulation: intensity

In the present work, I investigated how the visual system reacts to stimuli that cannot be perceived due to their low intensity. This low-intensity nature of the stimulus stands in contrast to a number of studies investigating subliminal stimulus *features*, for example stimuli flickering so fast that they cannot be perceived (Shady, MacLeod, & Fisher, 2004). While their features are imperceptible, the stimulus itself is visible and distinguishable from noise in the background. The subliminal stimuli used here, on the other hand, are not distinguishable from the background in the sense of signal detection theory, as explained above (Green & Swets, 1966).

2.5. The nature of the subliminal stimulation: comparison to masking

In typical metacontrast masking experiments, a suprathreshold, high intensity stimulus is made invisible by a second, spatially adjacent stimulus (the mask) that is at least comparable in physical strength or mask energy (Breitmeyer, 2006). That is, the target-stimulus would be in principle visible but has been made invisible by the mask. In these cases, numerous masking studies have shown effects of unconscious stimulus processing on perceptual, lexical and semantic levels (Kouider & Dehaene, 2007). Could masking occur when the mask is of lower contrast than the target? Specifically, could masking occur when the mask is of such low contrast that it cannot be perceived? I will discuss these questions in chapter 5.

Further, when comparing a visible target-stimulus to the same stimulus made invisible by masking, neural responses are often weaker, but similar (Harris, Wu, & Woldorff, 2011; Haynes & Rees, 2005). How does the visual system react to stimuli that are invisible due to their low contrast? I investigated these questions in an electrophysiological study (Study III).

2.6. Subliminal somatosensory stimulation and inhibition

Within the somatosensory system, it has been shown that low-intensity subliminal and high-intensity supraliminal stimuli lead to qualitatively different response patterns. Using functional Magnetic Resonance Imaging (fMRI), Blankenburg, et al. (2003) demonstrated that subliminal somatosensory stimulation, as compared to baseline, leads to blood-oxygenation-level-dependent (BOLD) signal deactivations in corresponding areas of the somatosensory cortex. Supraliminal somatosensory stimuli compared to baseline usually result in BOLD-signal increases. The BOLD-signal decrease in response to subliminal stimulation has been interpreted as focal deactivation due to selective activation of inhibitory interneurons. The function of this mechanism was suggested to entail the inhibition of noise. Indeed, a corresponding behavioural experiment showed that the perception of peri-liminal stimuli embedded in a train of subliminal stimulation was decreased, showing a functionally effective inhibition of the peri-liminal stimulus. Furthermore, another fMRI-experiment demonstrated reduced BOLD-signal responses to finger-stimulation by subliminal stimulation of an adjacent finger (Taskin, Holtze, Krause, & Villringer, 2008). Concurrently, the detectability of the target-finger stimulation is reduced by the subliminal stimulation of the adjacent finger.

Here, I investigated whether I can find similar inhibitory responses within the visual system using electroencephalography (EEG). Further, as has been found for somatosensory stimulation

(Blankenburg, et al., 2003, see above), I hypothesized that sensitivity thresholds of peri-liminal visual stimuli are increased when they are presented during subliminal stimulation.

2.7. Electrophysiology and inhibition: alpha-band power

Simultaneous recordings of fMRI and EEG have demonstrated an inverse correlation of the BOLD signal with alpha-band power (8-12 Hz) in multiple cortical areas, such as the occipital cortex (but not the thalamus). While BOLD-signal increases are associated with reduced alpha-band power BOLD-signal decreases are associated with elevated alpha-band power (Goldman, Stern, Engel, & Cohen, 2002; Moosmann, et al., 2003).

Furthermore, inhibitory responses have been correlated previously with BOLD-signal decreases using fMRI (as explained above), as well as with alpha-band increases using EEG. Elevated ongoing alpha-band power at stimulus onset inhibits processing of visual stimuli (Busch, Dubois, & VanRullen, 2009; Ergenoglu, et al., 2004), and selective attention increases alpha-band power in order to inhibit task-irrelevant stimuli (Busch & VanRullen, 2010; Foxe, Simpson, & Ahlfors, 1998; Klimesch, Sauseng, & Hanslmayr, 2007). By contrast, processing of supra-threshold or task-relevant stimuli is characterized by an event-related alpha-band power decrease (desynchronization) (Adrian & Matthews, 1934). Therefore, I hypothesized that subliminal visual stimulation – if it causes inhibition - leads to an alpha-band power increase.

2.8. Inhibition in the Elderly

With increasing age (above 50 years), humans gradually experience changes in vision such as decreased visual acuity and contrast sensitivity (Kline, Schieber, Abusamra, & Coyne, 1983; Ross, Clarke, & Bron, 1985). These visual declines are often due to changes in the optics of the eye (Spear, 1993). It is less well-known that some of these changes might be due to neurophysiological changes, as studies in macaque senescent V1 neurons suggest a degradation of intracortical inhibition with age (Schmolesky, Wang, Pu, & Leventhal, 2000). In humans, as explained above, low-intensity subliminal stimulation leads to inhibitory responses that are presumably due to a mechanism initiated by inhibitory interneurons in the somatosensory (Blankenburg, et al., 2003; Taskin, et al., 2008) as well as the visual system (Bareither, Chaumon, Bernasconi, Villringer, & Busch, 2014). I therefore hypothesized that the perception of visual target-stimuli during subliminal stimulation

would be decreased in a group of younger participants, but to a lesser extent in a group of elderly participants.

3. Research Questions and Hypotheses

- Subliminal stimulation inhibits visual perception of near-threshold target stimuli behaviourally.
- Inhibition of visual perception through subliminal stimulation is decreased in the elderly.
- Subliminal stimulation elicits elevated alpha-band responses, an index of cortical inhibition.

4. Summary of the Related Studies

4.1. Study I: Decreased visual detection during subliminal stimulation

Research Question

As has been shown previously within the somatosensory system, subliminal, not consciously detected stimuli, can influence perception: subliminal stimulation impairs perception of near-threshold stimuli and causes a function al deactivation in the somatosensory cortex (Blankenburg, et al., 2003; Taskin, et al., 2008). In this study, I investigated whether a similar behavioural inhibitory response exists within the visual system.

Design

A psychophysical study was conducted consisting of Phase I (threshold estimation), followed directly by Phase II (experimental test). In Phase I, each participant's absolute threshold for a stimulus train consisting of seven filled circular patches presented in a 7-Hz-rate was estimated. This was done in order to find a stimulus that was invisible for each individual participant. In order to ensure the subliminal nature, 25% of the absolute threshold in Phase II of the experiment was taken –further referred to as subliminal stimulus train. Second, each participant's 50% detection threshold for the target-stimulus was estimated, consisting of a single unfilled circle. The 50% detection threshold was taken as individual midpoint of the full psychometric function in Phase II of the experiment. In Phase II, target detection was tested for target-stimuli presented alone, target-stimuli embedded in a subliminal stimulus train on the same side, and target-stimuli presented on the opposite side of the stimulus train. The paradigm is illustrated in Figure 1.

Results and Discussion

Psychometric functions for the three conditions were fitted with a Weibull-function using the Palamedes Toolbox for Matlab (Prins, 2009) and are illustrated in Figure 2A. In order to analyze differences for the resulting 50% detection-thresholds between conditions, a repeated-measures analysis of variance (ANOVA) with condition (No-Train, Same-side, Opposite-side) as within-subject variable and resultant pairwise comparisons was conducted. Compared to targets presented alone, concurrent subliminal stimulation in the same hemifield resulted in a significantly increased

detection threshold. Subliminal stimulation in the opposite hemifield did not change the detection threshold (Figure 2A). Results for individual participants indicated that all participants showed elevated thresholds for target-stimuli presented at the same location as the subliminal train, whereas all but one individual indicated no effect for subliminal stimulation in the opposite hemifield (Figure 2B), thus confirming the previous results for individual participants.

To summarize, visual thresholds for target-stimuli were elevated during subliminal stimulation on the same side. These results are in analogy to previous results showing elevated thresholds for target-stimuli during somatosensory subliminal stimulation. The results are further discussed in chapter 5.

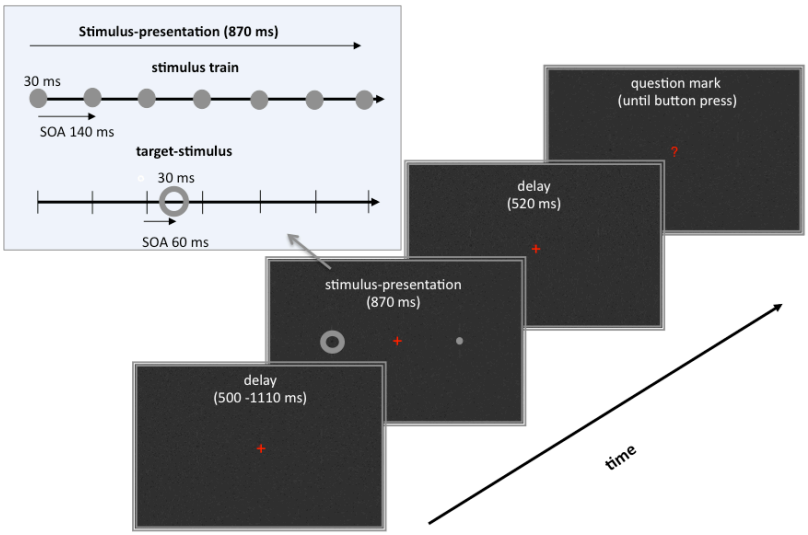

Figure 1 (Bareither, Villringer, & Busch, 2014)

Illustration of the paradigm during Phase II. Trials started with the presentation of a central fixation cross. After a variable delay, stimuli were presented in an interval of 870 ms. Illustrated is the Opposite-side condition – the stimulus train (illustrated by one out of seven patches, see insert) is presented in the right hemifield, the ring-shaped target-stimulus is presented in the left hemifield. The stimulus-presentation is followed by another delay of 520 ms. With the presentation of the question mark, participants were asked to indicate whether they saw a target-stimulus or not. **Stimulus-presentation (left insert).** The target-stimulus – non-filled circle - was presented either alone (No-Train condition), embedded in a subliminal stimulus train on the same side (Same-side condition), or presented on the opposite side of the subliminal stimulus train (Opposite-side condition). The subliminal train stimulus consisted of a sequence of seven filled patches. The target-stimulus was inserted after the third patch.

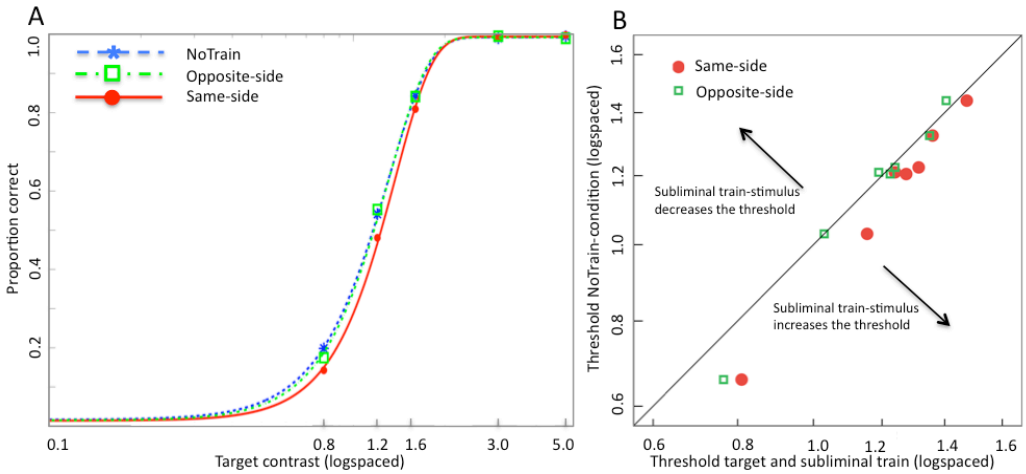

Figure 2. (Bareither, Villringer, et al., 2014)

A. Psychometric functions fitted to the grand-averaged data for target-stimuli when presented alone (NoTrain-condition; blue star), embedded in subliminal trains on the same side (Same-side condition; red dot) or embedded in subliminal trains on the opposite side (Opposite-side condition; green square). The log-spaced target contrast is expressed as proportions of the 50% detection threshold as determined in Phase I.
B. Thresholds for individual participants. No-Train condition (y-axis) plotted against the Opposite-side condition (green squares), as well as Same-side condition (red dots). The reference-diagonal indicates equal thresholds between conditions. The log-spaced target contrast is expressed as proportions of the 50% detection threshold as determined in Phase I.

4.2. Study II: Subliminal stimulation and inhibition of visual processing: investigating differences between young and elderly

Research Question

In Study I, it was shown that visual target-detection is decreased during subliminal stimulation in the same hemifield, as compared to target-stimuli presented alone or in the opposite hemifield in a group of younger participants (Bareither, Villringer, et al., 2014). It has been suggested previously that this inhibitory response might be mediated by intracortical inhibition (Blankenburg, et al., 2003; Taskin, et al., 2008). Correspondingly, primate studies investigating senescent V1 neurons suggest a degradation of intracortical inhibition with age (Schmolesky, et al., 2000). Therefore, I investigated differences in inhibitory responses between younger and elderly participants.

Design

The design of the study was identical to Study I. However, here, a group of seven younger (mean age 22.7, re-analysis of Bareither, Villringer, et al., 2014) and five elderly (mean age 70) participants was compared. All of the participants were tested in up to five sessions of the experiment, resulting in 19 sessions total for younger, and 20 sessions total for elderly participants. One session of the study consisted of Phase I (180 trials), followed directly by Phase II (1260 trials). Participants always took part in both phases during one session. Individual thresholds were estimated for a target-stimulus as well as a stimulus train (Phase I) that were presented in the experimental phase as target-stimulus in different intensities around the 50% detection-threshold and subliminal stimulus train (25% of the absolute threshold) respectively (Phase II). Target-detection was tested when targets were presented alone, embedded in a subliminal train on the same side, as well as embedded in a subliminal train in the opposite hemifield.

Results and Discussion

Psychometric functions for the three conditions were fitted with a Weibull-function using the Palamedes Toolbox for Matlab (Prins, 2009) and are illustrated in Figure 3. A mixed-design repeated measures ANOVA with age as between-subjects factor and condition as within-subject factor showed a significant main effect for condition but no main effect for age or an interaction between age and condition. These results were confirmed with a bootstrap technique implemented in the

Palamedes Toolbox. When both groups were analyzed separately, model comparisons revealed differences between all three conditions in both groups. Pairwise comparisons were similar for both groups, showing significantly different thresholds between the conditions in which the target was presented alone vs. presented during subliminal stimulation on the same side; as well as between targets presented during subliminal stimulation on the same side vs. subliminal stimulation in the opposite hemifield. For both age-groups, the difference between the conditions' targets presented alone vs. targets presented during subliminal stimulation in the opposite hemifield was not significant.

Results for individual participants are illustrated for young (Figure 4A) and elderly participants (Figure 4B). As indicated in Figure 4A, all young participants showed elevated thresholds for targets presented during subliminal stimulation on the same side (red dots). Thresholds for target-stimuli presented during subliminal stimulation in the opposite hemifield are close to the reference-line indicating no effect of subliminal trains for all but one participant. As indicated in Figure 4B, thresholds for three out of five elderly participants approach the reference line, indicating no effect of subliminal stimulation within the same hemifield (red dot). The distribution for target-stimuli with subliminal trains in the opposite hemifield shows most points falling on the reference line, indicating no effect of subliminal stimulation. The differences between young and elderly individual participants were not pronounced enough to lead to significant changes.

In summary, I could not find evidence for pronounced differences in inhibitory responses due to subliminal stimulation between young and elderly participants in the small sample used here. Different potential explanations exist and will be discussed in chapter 5

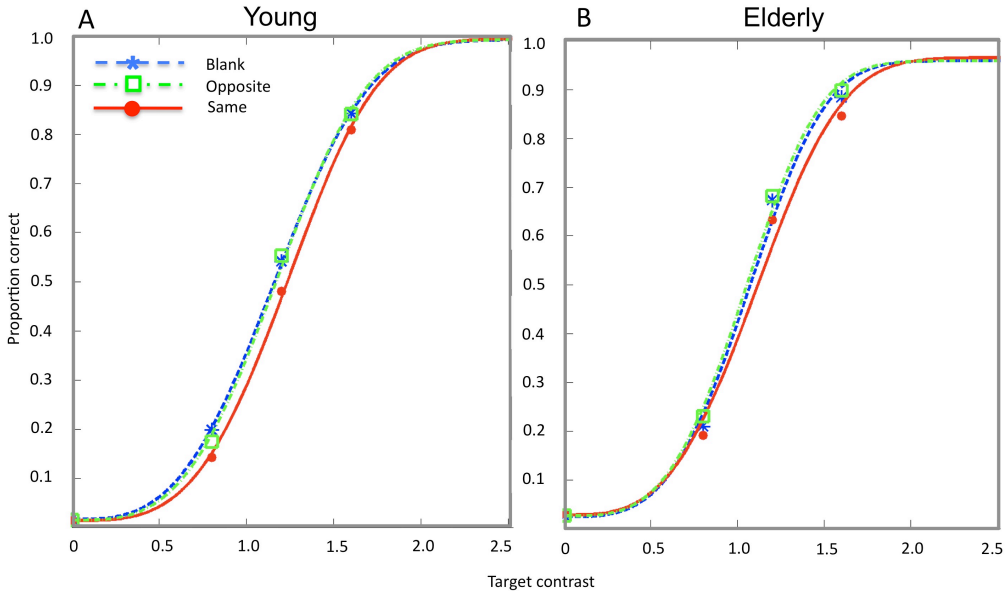

Figure 3: Fitted Psychometric Functions for target-stimuli when presented alone (blue star), embedded in subliminal trains on the same side (red dot) or embedded in subliminal trains on the opposite side (green square) for young (A) and elderly (B) participants. X-axis cut (between 2.5 and 5) for visualization purposes.

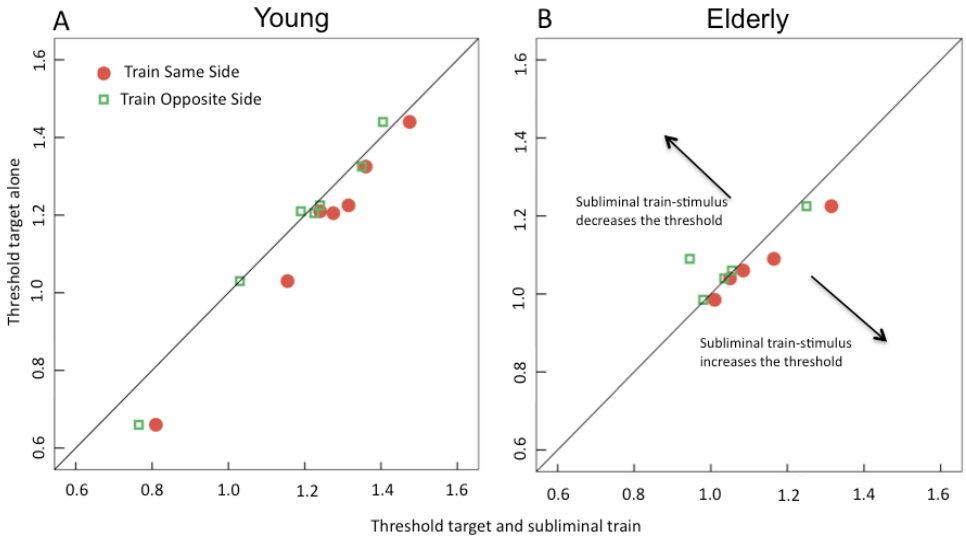

Figure 4. Thresholds for individual participants. No-Train condition (y-axis) plotted against the Opposite-side condition(green squares), as well as Same-side condition (red dots). The reference-diagonal indicates equal thresholds between conditions. The target contrast is expressed as proportions of the 50% detection threshold as determined in Phase

4.3. Study III: Invisible visual stimuli elicit increases in alpha-band power

Research Question

In the somatosensory system, low-intensity subliminal finger stimulation leads to a functional deactivation or inhibition in corresponding areas of the cortex (Blankenburg, et al., 2003). In Study III, I tested whether subliminal visual stimulation leads to an equivalent response within visual cortices, indicated by an increase in EEG alpha-band power.

Design

An EEG-experiment was conducted consisting of Phase I (threshold estimation) and Phase II (target detection task). In Phase I, each participant's absolute threshold for a single circular patch, presented peripherally for 30 ms was estimated. In Phase II, electrophysiological responses were recorded to subliminal stimuli (contrast set to 25 % of the participants individual sensitivity threshold as tested in Phase I) and supraliminal stimuli (contrast set to five times the individual threshold), as well as blank trials (no stimulation). Participants were asked to report circles that were randomly presented around the fixation cross. This task was introduced in order to keep the participants attention on the screen while electrophysiological responses were recorded to the peripheral subliminal and supraliminal stimuli. The paradigm is illustrated in Figure 5.

Results and Discussion

In this study, electrophysiological responses were recorded in response to visual stimuli that are invisible due to their low intensity and differentiated from those due to supraliminal stimulation. Our main analysis, a non-parametric test of oscillatory power (Maris & Oostenveld, 2007) revealed distinct activity patterns for paired comparisons of blank trials versus subliminal or supraliminal stimulation (Figure 6A). Compared to blank trials, supraliminal stimulation elicits a significant increase of lower-frequency power and a decrease that is maximal at the alpha-band power after 400 ms. This is a well-known response after visual stimulation (Adrian & Matthews, 1934). Subliminal stimulation elicits a significant increase with a maximum in the alpha-band around 300 ms. Compared to blank trials, the topography for supraliminal stimulation showed an occipital

desynchronization whereas subliminal stimulation revealed two maxima over occipital-parietal and left posterior channels (Figure 6A).

The phase-locking factor (Tallon-Baudry, Bertrand, Delpuech, & Pernier, 1996) of each condition was tested similarly to the analysis of the oscillatory power (Maris & Oostenveld, 2007) and revealed that only supraliminal stimuli showed significant phase-locking. There was no increase in phase-locking after presentation of subliminal stimulation, or during blank trials (Figure 6B).

The results are in line with predictions made from studies within the somatosensory system. Subliminal stimulation leads to BOLD-signal decreases within the somatosensory system as well as to alpha-band increases within the visual system. As has been shown above, both functional activations have been correlated with inhibitory mechanisms - possibly mediated through inhibitory interneurons. I will discuss this proposition in relation to the other studies in chapter 5.

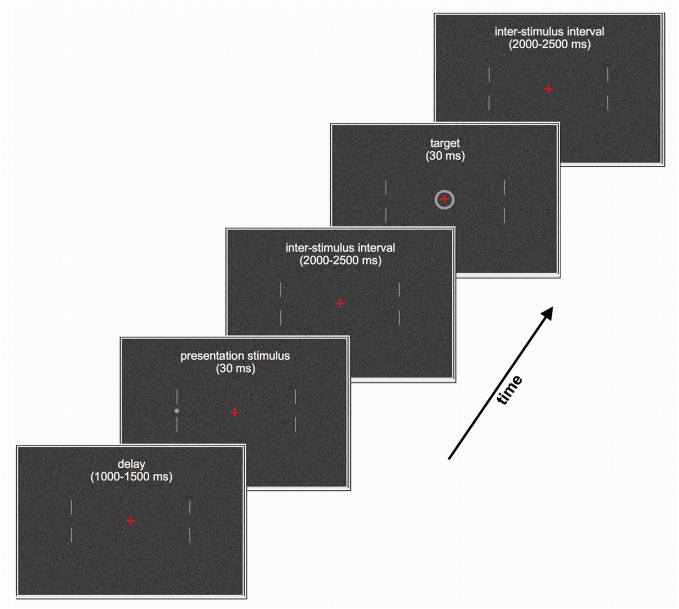

Figure 5 (Bareither, Chaumon, et al., 2014)

Illustration of the paradigm during Phase II (EEG recording). Trials started with the presentation of a fixation cross and peripheral markers. After a variable delay, stimuli were presented on 1/3 of the trials. The remaining were stimulus-absent blank trials. Participants were asked to count targets that were randomly interspersed at fixation between peripheral presentations.

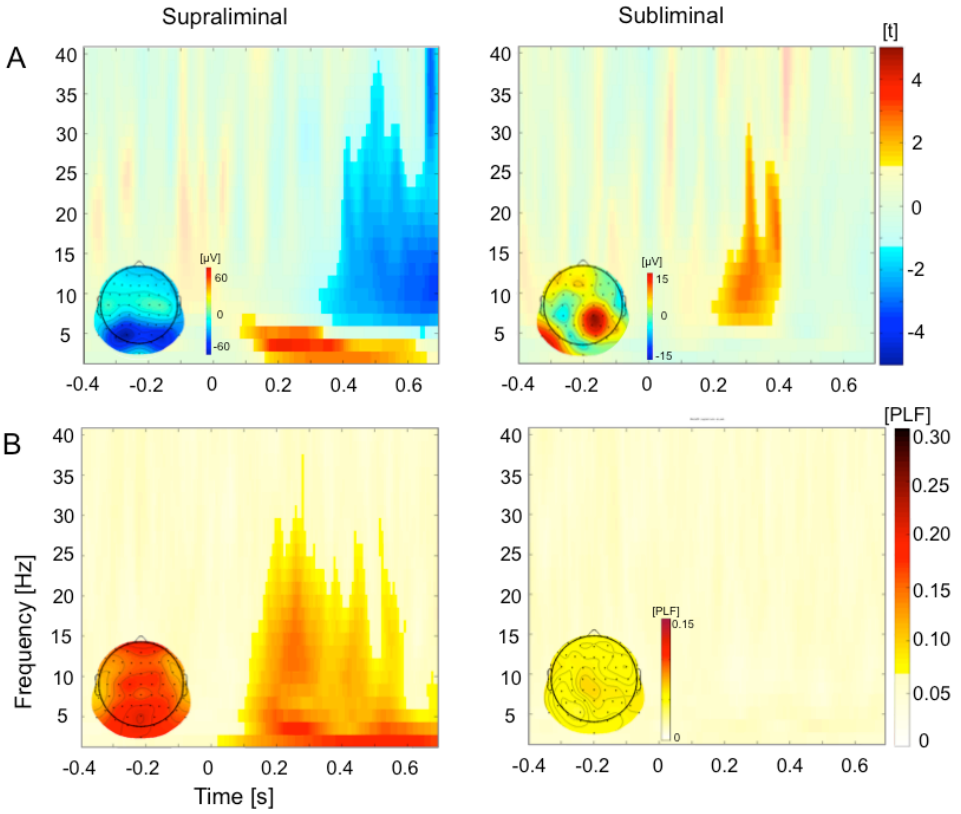

Figure 6 (Bareither, Chaumon, et al., 2014)

Analysis of oscillatory power. A. Time-frequency representation of event-related power changes averaged across channels with strongest alpha-power, showing oscillatory responses to supraliminal stimuli (left), as well as subliminal stimuli (right), as compared to blank trials. Non-significant regions are dimmed to improve visibility of the significant time-frequency clusters. Topographical distributions reveal an occipital desynchronization for supraliminal stimulation. Subliminal stimulation results in two maxima, synchronizing over parietal-occipital areas.

B. Time-frequency representation of the phase-locking factor (PLF) for supraliminal (left) and subliminal stimuli (right). Non-significant regions are dimmed to improve visibility of the significant time-frequency clusters. Note that the same analysis conducted on blank trials resulted also in no significant phase-locked response either (not shown). Topographies show the distribution of PLF-values within the alpha-frequency range (8-12 Hz; 200-400 ms).

5. General Discussion

In the present work, it could be shown that visual subliminal stimulation leads to inhibitory neural as well as behavioural responses. These results are in analogy with psychophysical and fMRI-studies showing inhibitory responses to subliminal somatosensory stimulation. Threshold sensitivity of peri-liminal stimulation of the index finger was reduced during subliminal stimulation of the same finger (Blankenburg, et al., 2003) as well as at a neighbouring finger (Taskin, et al., 2008). Similarly, here, it was shown that the threshold for target-stimuli during subliminal stimulation in the same hemifield increased significantly when compared to target-stimuli presented alone or presented in the opposite hemifield.

Within the somatosensory system, the behavioural responses were accompanied by a functional deactivation within somatosensory cortices, perhaps due to a focal inhibition mechanism mediated through cortical (Blankenburg, et al., 2003) or lateral inhibition (Taskin, et al., 2008). Similarly, results of the present study showed elevated alpha-band power in response to subliminal stimulation – an indicator for cortical inhibition.

Based on primate studies investigating senescent V1 neurons, it has been suggested that intracortical inhibition declines in elderly humans (see 2.8.). This decline is supposedly due to a degradation of inhibitory interneurons (Schmolesky, et al., 2000). In a study comparing inhibitory behavioural responses to subliminal stimulation in a group of elderly versus younger participants, we could not show a significant difference. Different explanations exist for this result.

Several explanations centre on the selective sample used in the current experiment. First, the number of participants in both groups is comparatively small. For young participants (Figure 4A), all but one of the individual thresholds for target-stimuli indicate no effect of subliminal stimulation on the opposite side. The distribution of thresholds during subliminal stimulation on the same side indicates a threshold-increase for all participants. For the elderly (Figure 4B), most of the individual thresholds for target-stimuli indicate no effect of subliminal stimulation on the opposite side. However, individual thresholds during subliminal stimulation on the same side indicate diminished inhibitory functioning in three out of five participants. This effect was not pronounced enough to lead to a significant interaction effect between the young and the elderly. On the other hand, using this set-up, it is not possible to exclude a tendency to diminished inhibitory response functioning. Future studies will require a larger sample size in order to clarify this issue.

Second, individuals signing up for experiments are also likely to engage with their environment more than other people their age and might therefore 'maintain' their brain better than other people their age - a finding termed 'Brain maintenance' (Nyberg, Lovden, Riklund, Lindenberger, & Backman, 2012). Due to individual differences, some participants show little or no age-related declines in cognitive functions. Engagement in socially, mentally and physically stimulating activities in old age helps keeping the brain functioning and cognitively performing well. Thus, individuals tested in this experiment might 'maintain' their brain-activity better than other persons of the same age.

Third, human participants tested in our study might have not been comparable in age to the senescent macaques used in the primate studies. These macaques were of an age equivalent to 90 years in humans, whereas our participants were between 65 and 75 years. Pronounced differences in inhibitory functioning might well be found in participants with increased age. This possibility was also raised by other studies, in which predictions from the same monkey-studies could not be confirmed in humans (Govenlock, Taylor, Sekuler, & Bennett, 2010).

5.1. Subliminal stimulation at the same side versus opposite side

A different explanation for our finding consists in the possibility that inhibitory responses seen in young and elderly in response to subliminal stimulation are not due to inhibitory interneurons as has been suggested before (Blankenburg, et al., 2003), but to other effects – such as cueing or masking. Considering the first possibility, detection performance in both behavioural studies was impaired for targets presented at the same location as the subliminal stimulus train, and unchanged for targets presented at the opposite side. This result indicates that subliminal trains did not act as temporal or attentional cues, such that target detection was affected by attention. Spatial attentional cueing should have resulted in improved performance for targets presented at the cued location, and impaired performance for targets presented at the uncued location in the opposite hemifeld, as compared to targets presented alone. Temporal cueing would have improved target detection regardless of the spatial locations of subliminal train and target.

5.2. Masking

The behavioural effect seen for both younger and elderly participants resembles that of metacontrast-masking. Generally, masking can be regarded as the decreased visibility of an object (the target) through the presentation of another object (the mask). In metacontrast-masking, the target-stimulus is masked through a surrounding stimulus that is at least comparable in physical strength or mask energy (Breitmeyer, 2006). In the present experiment, the inner subliminal patch would specify the mask, and the outer per-liminal ring would specify the target. Could theories of visual masking explain the reduced visibility of the outer peri-liminal target-ring by an inner "masking" subliminal stimulus trains?

Theories of visual processing assume that awareness occurs through a sustained and stable neural activation pattern, dependent on physical stimulus strength (Kiefer, et al., 2011). In current models of masking, it is often assumed that the neural activation pattern of a target-stimulus goes through feedforward processes into higher visual areas, but that feedback-mechanisms are disturbed by the fast procession of a disturbing stimulus (the mask), thereby leading to a mismatch between neural activation patterns (Enns & Di Lollo, 2000; Kiefer, et al., 2011; Lamme & Roelfsema, 2000). This model explains why masking-experiments systematically varying mask-target energy ratio (M/T) show that the physical strength (intensity) of the mask in relation to the target is directly related to the metacontrast-masking effect (Alpern, 1953). Based on this framework, a masking-effect due to a subliminal stimulus that is surrounded by the target (further decreasing its physical strength in comparison to the target) is expected to be small if at all present. By contrast, the effect size found in the present study indicates a rather strong effect. However, generally, the effects of masked high-intensity targets are difficult to compare to the periliminal target-stimuli used in this study.

I suggest that the effect seen in the present studies is not due to a mismatch between feedforward and feedback mechanisms, as suggested for masking. Rather, I propose that decreased perception of peri-liminal stimuli occurs through the activation of inhibitory interneurons that are preferentially activated through the subliminal stimulus. This activation of inhibitory interneurons leads to a downregulation of noise in relevant brain areas and inhibits the peri-liminal target-stimulus presented concurrently at the same retinal location. Further support for this notion comes from our electrophysiological study that is outlined below.

5.3. Alpha-band power and inhibition

In the third study of the present thesis, electrophysiological responses to subliminal stimuli were recorded and differentiated from the responses to supraliminal stimulation. When compared to blank trials, results showed a classic effect for supraliminal stimulation: a strongly phase-locked lower frequency increase likely to represent the event-related potential and an alpha-band power decrease (Adrian & Matthews, 1934). On the other hand, subliminal stimulation elicits a non-phase locked power increase that is maximal within the alpha-band (8-12 Hz) around 300 ms. Equivalent to BOLD-signal decreases within the somatosensory system, this specific neural activation pattern for subliminal stimulation suggests inhibitory response mechanisms.

Such a mechanism could be mediated through inhibitory interneurons. Physiological studies show that alpha-band power might subserve a "gating function" (Lopes da Silva, 1991), through hyperpolarization of thalamo-cortical interneurons and consequently a decreased probability to respond to external inputs (Foxe & Snyder, 2011; Jahnsen & Llinas, 1984). According to the "pulsed inhibition theory" (Jensen & Mazaheri, 2010), alpha-activity produces periods of inhibition that rhythmically reduce gamma activity, possibly due to GABA-ergic feedback from inhibitory interneurons (Lorincz, Kekesi, Juhasz, Crunelli, & Hughes, 2009). Additionally, feedforward inhibitory interneurons might be preferentially activated by subliminal stimulation: physiological studies in macaques have shown that inhibitory cortical interneurons have lower stimulation-thresholds compared to excitatory neurons in V1 (Zhuang, et al., 2013) as well as SI (Swadlow, 2003).

5.4. Function: inhibition of noise

The function of this inhibitory mechanism could be a protection against spurious channel noise in both the somatosensory (Blankenburg, et al., 2003; Taskin, et al., 2008) as well as the visual system (Bareither et al., 2014). Within the visual system, such a mechanism would be similar to automatic, unconscious inference-mechanisms that can be found in low-level visual processing (Rock, 1983). One instance of such an inhibitory inference mechanism is the "perceptual scotoma account" of motion-induced blindness (New & Scholl, 2008), in which target objects in full view fluctuate into and out of awareness when superimposed onto a global moving pattern (Bonneh, Cooperman, & Sagi, 2001; Wu, Busch, Fabre-Thorpe, & VanRullen, 2009). Accordingly, the visual system filters out input signals that are more likely due to imperfections of the eyeball or retina than to real events in the world. Thus, when visual input indicates a small object that is invariant with respect to salient,

global stimulus changes, the inference might be that this input is unlikely to result from a real object, to the effect that processing of the target is inhibited and the target object is not consciously perceived. Likewise, in the case of very weak, subliminal stimuli, the inference might be that the input signal is not of sufficient strength to indicate a real visual stimulus and momentarily inhibits further processing, including the processing of other stimuli at the same location.

5.5. Neural and behavioural consequences of subliminal stimulation

Previous studies have indicated that subliminal somatosensory stimuli cause a functional deactivation or inhibition in somatosensory areas. This deactivation was accompanied by reduced detection of peri-liminal stimuli when presented during subliminal stimulation. Here, results indicated equivalent cortical as well as behavioural responses within the visual system. While supraliminal stimulation leads to an early increase within lower-frequency bands and a decrease in alpha-band power from 400 ms on, subliminal stimulation leads to an alpha-band power increase around 300 ms. Further, I found increased detection thresholds for peri-liminal stimulation when presented during subliminal stimulation in the same hemifield. These responses could be found in elderly participants of up to 75 years.

While the finding of specific neural signatures in response to subliminal stimulation indicates neural processing of the stimulus, I suggest that the feedforward sweep is interrupted at a cortical level and no re-entrant process occurs, inhibiting conscious perception of the stimulus. The cortical down-regulation in response to the subliminal stimulus could serve as a suppression of input noise that has been described in the somatosensory system (Blankenburg, et al., 2003; Taskin, et al., 2008) and may well lead to the decreased detection of peri-liminal target-stimuli during subliminal stimulation.

6. Future Directions

With the present work, I could show inhibitory responses within the visual system using EEG that are in analogy to inhibitory responses shown within the somatosensory system using fMRI. However, it would be worthwhile to repeat the EEG-experiment within the visual system using fMRI. I would hypothesize to find BOLD-signal decreases within occipital areas similar to the areas found for supraliminal stimulation. Additionally, it remains to be seen whether the timing of the length of the subliminal stimulus (30 ms) as well as the timing of the inter-stimulus interval within the behavioural experiment are crucial for inhibitory mechanisms to occur. Similarly, it would be of

interest to investigate whether trains of subliminal visual stimuli lead to similar electrophysiological responses as single-stimuli and whether jittered vs. non-jittered stimulations lead to different responses. Another important step would be to further investigate the possibility of a masking-effect. First, it would be interesting to see whether a peri-liminal target-stimulus can be masked by a stimulus that is smaller in size than the target, but stronger in intensity. Also, it would be interesting to measure the sensitivity-threshold if target and mask do not share their outlines, e.g. if the peri-liminal surrounding target-stimulus is a square instead of a surrounding circle. If the decrease in sensitivity still persists under this circumstance, the effect is unlikely to be due metacontrast masking.

7. Final Conclusions

Walking along a dark street in the middle of the night, does our brain react similarly to the blinding light and the faint shadow? In this thesis, I could show that the brain reacts differently to a high-intensity supraliminal stimulus and a low-intensity subliminal stimulus. The blinding light of the motorcycle would likely lead to similar classic neural responses as the supraliminal stimulus presented within the electrophysiological study of this thesis: an increase within lower-frequency bands as well as a later decrease of alpha-band power.

Of all the faint shadows around us, filling the night with their different shades of darkness, there are some that we do not perceive consciously. As shown here, the brain processes these low-intensity stimuli in a way quite different from supraliminal stimuli. I suggest that the invisible shadow is processed but leads to a preferential activation of inhibitory interneurons leading to an interruption of the feedforward sweep and down-regulation of relevant areas. This results in the alpha-band increase seen in response to subliminal stimulation within the electrophysiological study of this thesis. The down-regulation could be due to a mechanism usually inhibiting noise within the visual system. Peri-liminal stimuli presented at the same location are similarly regarded as noise and processing of these is also inhibited, explaining the reduced threshold found in the psychophysical experiment presented here.

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Subliminal Stimulation and Inhibition of Visual Processing: Investigating Differences between Young and Elderly

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Abstract

With increasing age, humans experience changes in vision such as decreased visual acuity and contrast sensitivity. It has been known for a long time that these visual declines can be due to changes in the optics of the eye. Only recently was it suggested that some of these changes are due to neurophysiological changes, as studies in macaque sensescent V1 neurons show a degradation of intracortical inhibition. In humans, low-intensity subliminal stimulation elicits inhibitory responses, presumably due to intracortical inhibition. We investigated these inhibitory responses in a group of younger and elderly participants. We could not find evidence for pronounced differences between the two groups and discuss different potential explanations.

1. Introduction

Elderly humans (above 50 years) experience changes in vision such as decreased visual acuity and contrast sensitivity (Kline, Schieber, Abusamra, & Coyne, 1983; Spear, 1993) that can be due to changes in the optics of the eye (Spear, 1993). In recent years, evidence from primate-studies accumulated into the proposition that vision in the elderly may be partly degraded through neural changes in the visual cortex (Schmolesky, Wang, Pu, & Leventhal, 2000; Yu, Wang, Li, Zhou, & Leventhal, 2006; Zhang, et al., 2008). While striate and extrastriate cells in young monkeys exhibit strong direction- and orientation-selectivity, cells in senescent monkeys exhibit decreased orientation and direction selectivity, generally increased visual responsiveness, and increased spontaneous activity. It has been suggested that primate visual cortical function declines because of degraded inhibitory intracortical connections that accompany senescence (Schmolesky, et al., 2000). Indeed, after administration of gamma–aminobutyric acid (GABA) - the main inhibitory neurotransmitter in the brain – visual function of senescent cells improved to a level comparable to young cells (Leventhal, Wang, Pu, Zhou, & Ma, 2003).

In humans, low-intensity subliminal stimulation leads to inhibitory responses that are presumably due to inhibitory interneurons in the somatosensory (Blankenburg, et al., 2003; Taskin, Holtze, Krause, & Villringer, 2008) as well as in the visual system (Bareither, Chaumon, Bernasconi, Villringer, & Busch, 2014). Within the somatosensory system, low-intensity subliminal stimulation leads to blood oxygenation level-dependent (BOLD)-signal decreases, while supraliminal stimulation usually leads to BOLD-signal increases. This response has been interpreted as a focal inhibition mechanism that protects the cortex from noise. A corresponding behavioural experiment

supported this notion by showing increased detection-thresholds for near-threshold stimuli during subliminal stimulation as compared to near-threshold stimuli presented with no additional stimulation (Blankenburg, et al., 2003).

In analogy to these studies, we could previously show that visual subliminal stimulation leads to functional responses that have been correlated to inhibitory mechanisms (Bareither, Chaumon, et al., 2014). By using Electroencephalography (EEG), supraliminal stimulation resulted in an early increase of lower frequencies (likely reflecting the event-related potential) as well as a late decrease of alpha-band power (8-12 Hz), while subliminal visual stimulation caused an increase in alpha-band power around 300 ms. Increases in alpha-band power have been correlated previously to inhibitory mechanism. Elevated ongoing alpha-band power at stimulus onset inhibits processing of visual stimuli (Busch, Dubois, & VanRullen, 2009; Ergenoglu, et al., 2004), and selective attention increases alpha-band power in order to inhibit task-irrelevant stimuli (Busch & VanRullen, 2010; Foxe, Simpson, & Ahlfors, 1998; Klimesch, Sauseng, & Hanslmayr, 2007). By contrast, processing of supra-threshold or task-relevant stimuli is characterized by an event-related alpha-band power decrease (desynchronization) (Adrian & Matthews, 1934). Furthermore, simultaneous recordings of functional Magnetic Resonance Imaging (fMRI) and EEG have demonstrated an inverse correlation of the BOLD signal with alpha-band power (8-12 Hz) in multiple cortical areas, such as the occipital cortex (Goldman, Stern, Engel, & Cohen, 2002; Moosmann, et al., 2003).

Together, these results support the notion of inhibitory cortical mechanism in response to subliminal stimulation, as suggested by Blankenburg et al. (2003). The function of such a mechanism could be a protection of the visual system against weak stimulation that is unlikely to be caused by real events in the world. In young participants, subliminal stimulation might lead to noise suppression through intracortical inhibition mediated through inhibitory neurotransmitters, such as GABA. At the same time, intracortical inhibition is thought to mediate the strong orientation and direction selectivity, decreased visual responsiveness and decreased spontaneous activity of cells observed in young vs. senescent monkeys (see above). Decreased orientation selectivity and increased visual responsiveness in cells of senescent monkeys is correlated with decreased intracortical inhibition.

In this study, we investigated the influences of subliminal stimulation on the perception of near-threshold target-stimuli in young and elderly adults. Sensitivity thresholds for target-stimuli were increased during subliminal stimulation on the same side for both young and elderly participants. We did not find pronounced differences between the two groups.

The following methods are similar to Bareither, Villringer, & Busch (2014)

2. Materials and Methods

The purpose of this study was to investigate the detectability of target-stimuli when they were embedded in a train of subliminal stimuli in a group of young as compared to elderly participants. In Phase I of the experiment, we estimated each participant's visual sensitivity threshold for the target-stimulus as well as the stimulus train. In Phase II, we tested performance in a target detection task.

2.1. Participants

Seven young participants (re-analysis of Bareither, Villringer, & Busch, 2014) and six elderly participants (age 65-75, mean age 70, 5 females, 6 right-handed) took part in this experiment. Participants in both groups were tested in up to five sessions of the experiment, resulting in 19 sessions total for younger, and 20 sessions total for elderly participants. One session of the study consisted of Phase I (180 trials), followed directly by Phase II (1260 trials). Participants always took part in both phases during one session.

All participants reported no history of neurological or psychiatric disorders and normal or corrected-to-normal vision. Informed consent was obtained from each participant after explanation of the study. The experiment was approved by the Ethics Committee of the Charité-Universitätsmedizin Berlin, according to the Declaration of Helsinki.

Elderly participants were screened for cognitive impairment with the Mini Mental State Examination (MMSE, M=29, SD=0.63, range = 28 – 30). To ensure the age-typicality of our samples, both groups were tested on fluid intelligence - Digit Symbol, WAIS-III (Wechlser, 1997) and crystallised intelligence – verbal Knowledge, MWT-B (Lehrl, 1977). Results showed a typical age-related decrease in perceptual speed ($t(11) = 4.08$, p=0.0018 and increase in verbal knowledge ($t(11) = 2.63$, p=0.0234). Mean educational levels were 13.8 (SD=3.8) years for younger adults and 14 (SD=1.4) years for older adults. Visual acuity was tested using Landolt-Rings with a standard distance of 6m. The cut-off was set at 20/40 (participant needs twice the size as normal-seeing person) for distant vision. Close vision was tested in Phase I of the experiment (see below). Participants who detected less than half of the stimuli of the highest intensity, were excluded from further analysis. Additionally, two of the elderly participants that were tested repeatedly did not see more than half of

the stimuli of the stimuli with the highest intensity in one run each. These runs were also excluded from further analysis.

2.2. Stimulus and Apparatus

The experiment was programmed in Matlab (Mathworks, Inc.) using the Psychophysics Toolbox (Brainard, 1997). Participants were seated in a dark, sound-attenuated chamber. Stimuli were presented on a gamma-linearized 19″ CRT monitor with 1280x1024 resolution and refresh rate of 100 Hz, located 56 cm from the participants' eyes. Head position was stabilized using a chin rest. For all phases of the experiment, the background consisted of a spatially modulated random white noise pattern. Mean luminance of the background pattern was 10.76 cd/m^2. A central red fixation cross 0.63◦ in diameter was continuously present on the screen. The target-stimulus was an unfilled circle (ring), 0.76◦ in diameter (inside diameter 0.39◦), presented once for 30 ms. The stimulus train consisted of seven consecutive filled circular patches, each 0.38◦ in diameter. The patches were presented at a 7Hz-rate for 1000 ms. Each of the seven patches was presented for 30 ms with an interstimulus-interval of 110 ms. Both target-stimuli and train-stimuli were presented on the horizontal meridian at an eccentricity of 18.3◦ to the right or the left of the fixation cross. Participants were instructed to always maintain central fixation. In one session of the experiment, participants were tested in Phase I (threshold estimation) followed directly by Phase II (experimental test).

2.3. Procedure

2.3.1. Phase I – Contrast-Threshold Estimation

Stimulus contrasts were determined individually for each participant to account for inter-individual differences in sensitivity. In the first phase of the experiment, we estimated each participant's absolute sensitivity threshold for the stimulus train, as well as the 50% performance threshold for the target-stimulus. This was done in order to find individual thresholds that could be used in the directly following Phase II of the experiment. Finding the absolute threshold for train stimuli was important in order to create a stimulus that was invisible for each individual on every trial. The purpose of the 50% detection threshold for target stimuli was to find a contrast that could serve as individual midpoint of the full psychometric function, which encompasses stimulus contrasts ranging

from virtually invisible to clearly visible.

Each trial started with the presentation of a fixation cross, which was continuously present on the screen for the whole trial. After a variable delay (range: 1 to 1.5 s), stimuli were presented in 87.5% of the trials for each condition. The remaining trials were stimulus-absent "catch" trials to estimate the false-alarm rate. After another delay of one second, the fixation cross turned into a question mark. With the presentation of the question mark, participants were asked to indicate via button press whether or not they saw any stimulus – be it a target-stimulus or a stimulus train. Each trial of the experiment consisted of either a stimulus train or a target-stimulus, chosen randomly from the set of intensities. Stimuli were presented at seven different intensities (stimulus train: 11.5, 12.0, 12.5, 13.0, 13.5, 14.5, 15.0 cd/m^2; target-stimuli: 11.0, 12.3, 12.8, 13.0, 13.2, 14.5, 15.5 cd/m^2), 20 repetitions per intensity (plus 20 repetitions for "catch trials". The intensities were based on pilot-experiments.

2.3.2. Phase II – Target-stimulus Detection Task

In Phase II, we tested performance in a target detection task for target-stimuli presented alone (No-Train condition), target-stimuli embedded in a stimulus train on the same side (Same-side condition), and target-stimuli presented on the opposite side of the stimulus train (Opposite-side condition).

To ensure that the stimulus train was really subliminal, the contrast of the stimulus train (as estimated individually in Phase I) was set to 25% of the participant's absolute sensitivity threshold. The target-stimuli were presented in six different intensities distributed around the 50%-threshold (0, 0.8, 1.2, 1.6, 3 and 5 times the threshold as calculated in Phase I), with 70 repetitions per intensity. As in Phase I, trials were initiated via button press and started with the presentation of a fixation cross, which was from then on presented continually on the screen. After a variable delay (500-1100 ms), the stimulus train (if applicable, see below) and the target stimulus (if applicable, see below) were presented during an interval of 870 ms. After another delay of 520 ms (during which only the fixation cross was presented), the fixation cross turned into a question mark, and participants reported whether or not they detected a target-stimulus (detection of trains was not required in Phase II).

The stimulus train was presented on 2/3 of the trials, either on the same side as the target-stimulus (Same-side condition) or on the opposite side of the target-stimulus (Opposite-side condition). For example, if the subliminal stimulus train was presented on the left, the target stimulus would be

presented on the left as well (Same-side condition) or on the right (Opposite-side condition). The location of the subliminal train stimulus was chosen randomly. On the remaining 1/3 of trials, only target-stimuli were presented (No-Train condition), but the background was displayed for the same duration as in the other conditions, such that trial durations were identical in all conditions (see Figure 1).

Following the third patch of the stimulus train, we interleaved the target-stimulus after a delay of 30 ms - similarly to Blankenburg et al. (2003). The target-stimulus was presented on 83.33% of the trials. The rest of the trials were stimulus-absent trials, used to estimate the false-alarm rate.

The stream of 1260 trials in total was interrupted by five breaks, separating the experiment into six blocks. Participants could start the next block manually.

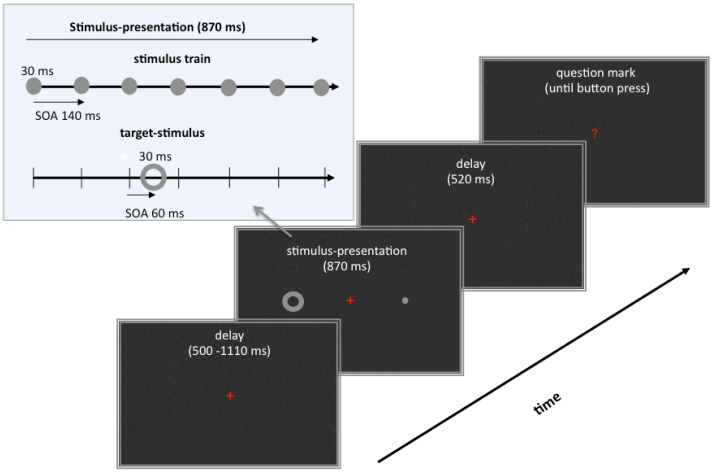

Figure 1. Illustration of the paradigm during Phase II. Trials started with the presentation of a central fixation cross. After a variable delay, stimuli were presented in an interval of 870 ms. Illustrated is the Opposite-side condition – the stimulus train (illustrated by one out of seven patches, see insert) is presented in the right hemifield, the ring-shaped target-stimulus is presented in the left hemifield. The stimulus-presentation is followed by another delay of 520 ms. With the presentation of the question mark, participants were asked to indicate whether they saw a target-stimulus or not.

Stimulus-presentation (left insert): The target-stimulus – non-filled circle - was presented either alone (No-Train condition), embedded in a subliminal stimulus train on the same side (Same-side condition), or presented on the opposite side of the subliminal stimulus train (Opposite-side condition). The subliminal train stimulus consisted of a sequence of seven filled patches. The target-stimulus was inserted after the third patch.

2.4. Data Analysis

In Phase I of the experiment, we estimated thresholds for the stimulus train as well as the target-stimulus separately. To determine individual absolute sensitivity thresholds for the stimulus train, we calculated D-prime (d′) of each of the different stimulus intensities using the participant's hit rate and false alarm rate for that intensity. We then simulated the performance of an observer with zero sensitivity in a detection task with the same number of signal trials and catch trials as in the real experiment. This simulation was repeated 100,000 times, yielding a distribution of expected d′ values for an observer with zero sensitivity. This allowed us to compute for each of the different stimulus intensities the probability that a participant's empirical d′ was achieved despite zero sensitivity. A participant's absolute sensitivity threshold for train stimuli was defined as the minimal stimulus intensity required for a d′ for which this probability was < 0.01.

To determine the 50%-threshold for target-stimuli (i.e. the contrast required for 50% correct performance), we fitted the data (number of yes-responses for each stimulus-intensity) with a Weibull-function, using the Palamedes Toolbox for Matlab (Prins, 2009). Psychometric functions were fitted by estimating the parameters α (threshold), β (the slope), γ (the guess-rate) and λ (the lapse-rate). In this part of the experiment, initial guesses of Weibull function parameters γ (the guess-rate) and λ (the lapse-rate) were given according to the individual responses for the guess-rate and the lapse-rate.

As in Phase I, the 50% threshold for target detection in Phase II was determined by fitting a Weibull-function to the averaged data for each of the three conditions (No-Train, Same-side and Opposite-side) using the Palamedes Toolbox for Matlab. In Phase II, initial guesses of Weibull function parameters (1, 3, 0.01, 0.01) were the same for all participants. Parameters were free to vary, with the constraint that lapse- and guess-rate varied between 0 and 0.1.

In order to analyze differences for the resulting 50%-thresholds between conditions and age, we conducted a repeated-measures analysis of variance ANOVA with age (Young and Elderly) as between-subject variable and condition (Blank, Same, Opposite) as within-subject variable. Additionally, we analyzed differences between the conditions for the Grand Average over young and elderly participants, as well as for the groups separately, using a bootstrap technique implemented in the Palamedes Toolbox. First, the number of yes-responses for individuals that conducted several sessions were averaged across all trials. Then the number of yes-responses was summed across individuals and psychometric functions were fitted as described above for each of the three conditions. The resulting parameters from the fit were given as estimates to a function comparing a

model with different thresholds to a model with identical thresholds ('PAL-PFLR_ModelComparison'). The likelihood ratios for the observed data were calculated for the comparison between the threshold-identical (constrained) model and the threshold-different (unconstrained) model. If the number of yes-answers differs between the conditions, the unconstrained model will have a higher likelihood than the constrained model and the likelihood ratio (constrained/unconstrained model) will be < 1. The smaller the likelihood ratio, the more likely becomes the unconstrained model in comparison with the constrained model. To estimate whether the resulting likelihood ratio is due to chance or reflects a real difference between the conditions, a random observer was simulated acting according to the constrained model via Monte-Carlo simulations. We performed 5000 simulations for model comparisons between all three conditions, first for the average over all participants, then for young and elderly separately. Since the analysis resulted in a $p<0.01$, we performed pairwise comparisons between each pair of the conditions.

3. Results

3.1. Phase I

We estimated each participant's absolute threshold for the stimulus train as well as the 50% detection threshold for the target-stimulus in Phase I of the experiment. An independent-samples t-test between subjects' threshold-means revealed the expected significant difference in 50%-threshold between young (M = 0.1764, SD = 0.0042, or 12.8 cd/m^2) and elderly (M = 0.2003; SD = 0.006, or 14.4 cd/m^2) for the target-stimulus ($t(10) = -0.8165$, $p<0.001$). However, we could not find a significant difference for the absolute threshold between young (M = 0.1688, SD = 0.0051, corresponding to 12.2 cd/m^2) and elderly (M = 0.1763, SD = 0.0099, corresponding to 12.8 cd/m^2) for the stimulus train ($t(10) = -1.73$, $p = .11\ 5$).

Since this result was unexpected, we elaborated whether it was due to the different thresholds analyzed (50%-threshold vs. absolute threshold) or whether it was due to the nature of the stimuli used (train vs. single stimulus). First, we tested whether the absolute thresholds of the stimulus train and target-stimulus are comparable. Analysis revealed very similar results for the young participants absolute threshold of the stimulus train (M = 0.168, SD = 0.0051) and the target-stimulus (M = 0.164, SD = 0.0067). However, the thresholds for the elderly differed between the stimulus train (M = 0.176, SD = 0.0099) and the target-stimulus (M = 0.182, SD = 0.0095). Again, while the

difference in age for the stimulus train did not reach significance ($t(10) = 1.725$; $p = 0.115$), the age-difference for the target-stimulus was significant ($t(10) = 3.56$; $p = 0.005$). The expected disadvantage of elderly participants due to the aging visual system seems to be attenuated by the presentation of a train of stimuli.

Individual absolute thresholds for the stimulus train were used in Phase II of the experiment. To ensure the subliminal nature of the stimulus train, their luminance was set to 25% of the absolute threshold as estimated in Phase I.

3.2. Phase II

By using a Maximum Likelihood criterion, psychometric functions could be fitted successfully to all participants but one. This elderly participant had an extremely high bias towards yes-responses (for trials in which no stimulus was presented, up to 27 yes-responses in 70 trials, as averaged over 3 runs) and was therefore excluded from further analysis.

Results of a mixed-design repeated measures ANOVA with age as between-subjects factor and condition as within-subject factor revealed a significant main effect for condition ($F(2,20)=11.15$; $p<0.001$; $\eta_p^2=.53$) indicating increased sensitivity threshold for target-stimuli during subliminal stimulation on the same side, but no main effect for age ($F(2,20)=0.9$, $p=0.4$, $\eta_p^2=0.08$) or an interaction between age and condition ($F(2,20)=1.01$, $p=0.4$, $\eta_p^2=0.09$).

Our main hypothesis concerned differences in the effects of target-stimulus presented alone versus target-stimulus presented during subliminal stimulation on the same side between young and elderly participants. To test this hypothesis directly, we calculated individual difference scores of the two conditions for each individual participant. An independent-samples t-test of subjects' difference-scores between young and elderly was not significant ($t(10)=0.37$; $p=0.72$).

Further, we investigated our results with an alternative method, a bootstrap technique implemented in the Palamedes Toolbox (see Methods). We compared a model in which thresholds between the conditions are identical (constrained model), to a model in which thresholds are allowed to differ (unconstrained model). The psychometric function fitted to the summed individual averages of all participants is shown in Figure 2. When data of both groups were summed, the likelihood-ratio test indicated that a model in which thresholds differ between all three conditions was more adequate for modelling the data than a constraint model that assumes no condition differences ($p < 0.001$).

Pairwise comparisons showed significantly different thresholds between the conditions No-Train vs. Same ($p < 0.001$) and opposite vs. Same ($p < 0.001$), but not for No-Train vs. Opposite ($p = 0.68$).

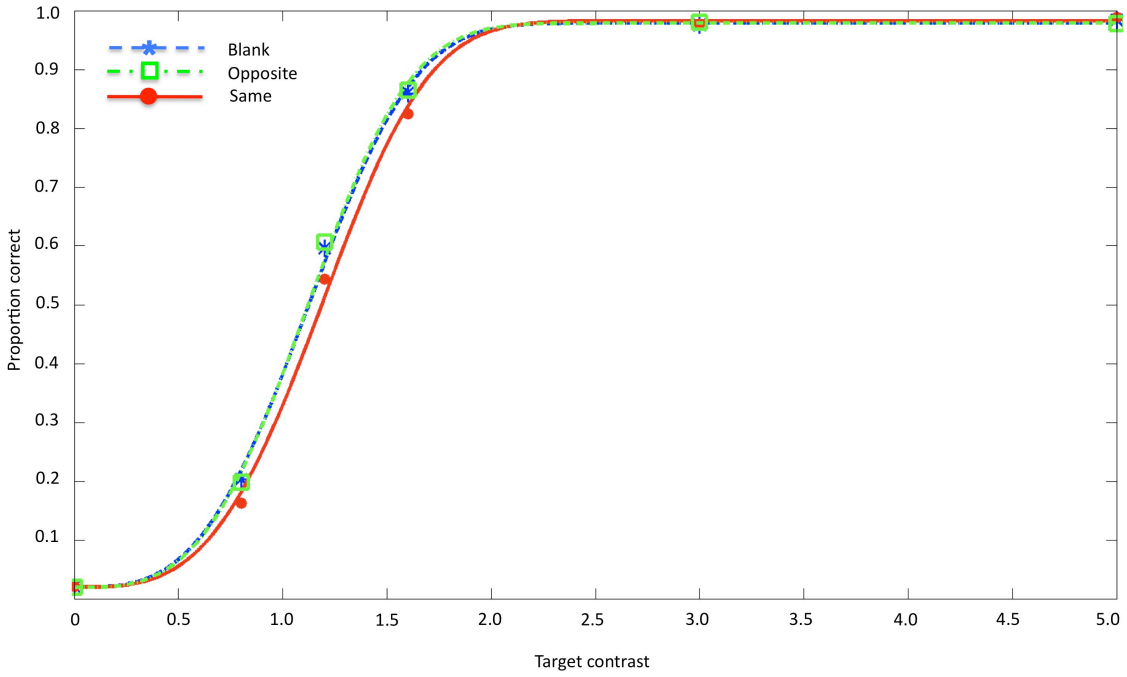

Figure 2. Psychometric functions fitted to the grand-averaged data over both groups for target-stimuli when presented alone (NoTrain-condition; blue star), embedded in subliminal trains on the same side (Same-side condition; red dot) or embedded in subliminal trains on the opposite side (Opposite-side condition; green square).
The target contrast is expressed as proportions of the 50% detection threshold as determined in Phase I.

Similar results were found when the groups were analysed separately. For young participants (Figure 3A), model comparisons revealed significant differences between all three conditions ($p < 0.01$). Pairwise comparisons showed significantly different thresholds between the conditions No-Train vs. Same ($p = 0.01$) and Opposite vs. Same ($p < 0.01$), but not for No-Train vs. Opposite ($p = 0.77$). Similarly for the group of elderly participants (Figure 3B), assuming identical thresholds between all three conditions is highly unlikely ($p<0.01$), as well as for thresholds between Blank vs. Same ($p < 0.05$) and Same vs. Opposite ($p = 0.01$), but not for Blank vs. Opposite ($p = 0.43$).

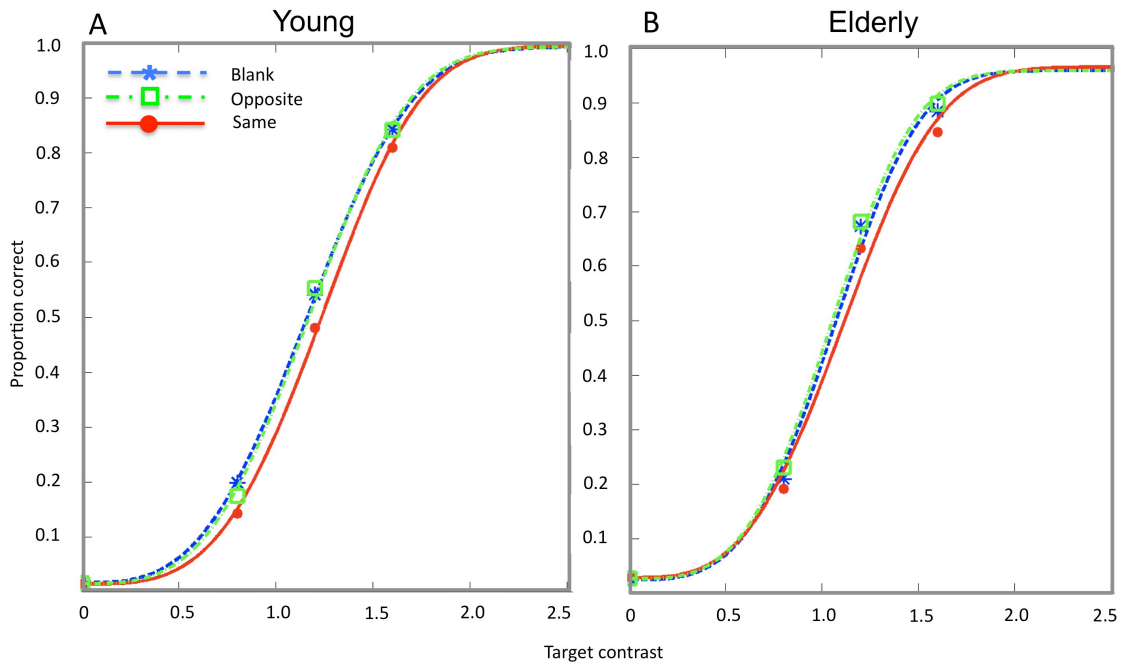

Figure 3: Fitted Psychometric Functions for target-stimuli when presented alone (blue star), embedded in subliminal trains on the same side (red dot) or embedded in subliminal trains on the opposite side (green square) for young (A) and elderly (B) participants. X-axis cut (between 2.5 and 5) for visualization purposes.

Results for individual participants are illustrated for young (Figure 4A) and elderly participants (Figure 4B). Detection thresholds for target-stimuli presented alone (No-Train-Condition; y-axis) are plotted against thresholds for target-stimuli presented with concurrent trains of subliminal stimuli, either at the same location (red dots) or in the opposite hemifield (green squares). In this image, points falling on the reference-diagonal indicate equal thresholds between the conditions, i.e. no effect of subliminal trains. Considering the distribution of individual thresholds for the group of younger participants, thresholds for target-stimuli during subliminal stimulation in the opposite hemifield are very close to this line. The participant showing the lowest threshold completed Phase II with an unusually high detection-rate in all three conditions. To test whether our results are sensitive to the unusually high detection rate of this subject, we performed the analysis with and without this subject. The findings are robust, i.e., the main findings did not change whether the subject was included or excluded in the analysis. By contrast, all participants showed elevated

thresholds for targets with subliminal trains at the same location, as indicated by the fact that all points were located below the reference diagonal.

The distribution for target-stimuli with subliminal trains in the opposite hemifield is similar for elderly participants, with most points falling very close to the reference line. However, considering targets with subliminal trains at the same location, three out of five individual thresholds approach the reference line closely.

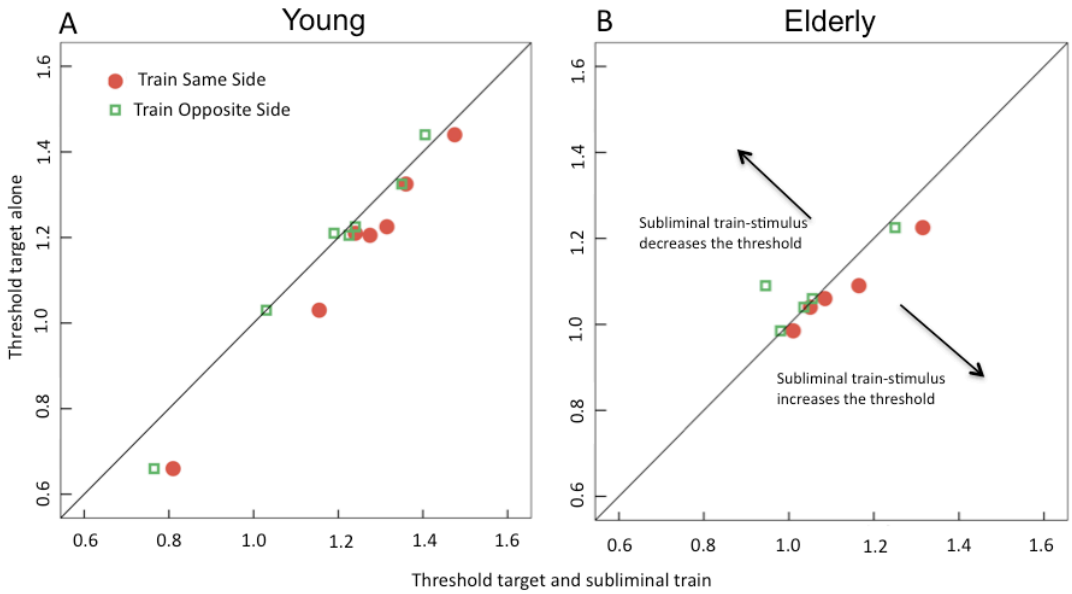

Figure 4. Threholds for individual participants. No-Train condition (y-axis) plotted against the Opposite-side condition(green squares), as well as Same-side condition (red dots). The reference-diagonal indicates equal thresholds between conditions. The target contrast is expressed as proportions of the 50% detection threshold as determined in Phase I.

4. Discussion

We investigated influences of subliminal stimulation on the perception of near-threshold visual target-stimuli in young and elderly adults. When compared with a condition in which peripheral targets were presented alone, we found significantly increased thresholds for visual stimuli that were presented during subliminal stimulation on the same side for both age-groups. Subliminal

stimulation in the opposite hemifield did not lead to any significant changes in either group. We could not find pronounced differences between the groups.

When humans get older, their visual abilities can change substantially. With increasing age (above 50 years), a possible wide range of visual deficits includes decreased visual acuity and contrast sensitivity, as well as increased response-times in visual tests (Kline, et al., 1983; Spear, 1993). While some of the visual declines might stem from changes in the optics of the eye (presbyopia; senile miosis), there is accumulating evidence of neural changes, specifically the striate cortex (for a review see Spear, 1993). While the majority of studies of human as well as macaque striate cortex found no decrease in neuron density during aging, studies in primates show that cells in V1 and V2 exhibit decreased orientation and direction selectivity, increased visual responsiveness and increased spontaneous activity (Schmolesky, et al., 2000; Yu, et al., 2006), as well as a decline in spatial and temporal sensitivities (Zhang, et al., 2008). The authors suggest that these changes might be due to degraded inhibitory intracortical connections that accompany senescence. Indeed, administering GABA, the main inhibitory neurotransmitter in the brain, reversed the effects of aging (Leventhal, et al., 2003). The administration of GABA as well as its agonist muscimol resulted in improved orientation and direction selectivity, decreased visual responsiveness and decreased spontaneous activity. It has been proposed that visual changes seen in humans during senescence might therefore be due to degraded inhibitory connections (Leventhal, et al., 2003; Schmolesky, et al., 2000; Yu, et al., 2006; Zhang, et al., 2008).

Concurrently, inhibitory cortical interneurons in primary somatosensory, as well as primary visual cortex have lower stimulation-thresholds compared with excitatory neurons (Swadlow, 2003; Zhuang, et al., 2013). Weak, subliminal stimulation might therefore favour activation of feedforward inhibitory interneurons. This proposition has been made for younger human participants in the somatosensory (Blankenburg, et al., 2003; Taskin, et al., 2008) as well as the visual system (Bareither, Chaumon, et al., 2014). Blankenburg, et al. (2003) have shown that subliminal somatosensory stimulation not only leads to a negative BOLD signal in SI, SII, and SMA, but was also associated with an elevated threshold for additional sensory stimuli as well as an attenuated BOLD response to subsequent supraliminal stimuli. The effect may be related to lateral inhibition, since Taskin, et al. (2008) showed reduced perception of finger stimulation during subliminal stimulation of an adjacent finger. This finding was also accompanied by a reduced BOLD-response of the finger stimulation when an adjacent finger was stimulated subliminally. Moreover, by using EEG, alpha-band power increases have been shown in response to subliminal stimulation in the

visual system (Bareither, Chaumon, et al., 2014) indicating reduced activity of the respective sensory cortical area (Haegens, Nacher, Luna, Romo, & Jensen, 2011). Furthermore, simultaneous recordings of fMRI and EEG have demonstrated an inverse correlation of the BOLD signal with alpha-band power (Goldman, et al., 2002; Moosmann, et al., 2003). Also, increased alpha-band power at stimulus onset has been found to inhibit processing of visual stimuli (Busch, et al., 2009; Ergenoglu, et al., 2004), and alpha-band power was increased when task-irrelevant stimuli are inhibited during selective attention (Busch & VanRullen, 2010; Foxe, et al., 1998; Klimesch, et al., 2007). Thus, increased alpha-band power in response to subliminal stimulation is interpreted as an inhibitory response in analogy to BOLD-signal decreases.

Here, we tested effects of subliminal stimulation on near-threshold target-stimuli presented in a group of younger and elderly adults. We could not show significant differences between the two groups. Different potential explanations exist for this result.
First, the effect of decreased perception during subliminal stimulation could be due to a different mechanism than the proposed cortical inhibition. On a behavioural level, the effect resembles the well-known phenomenon of masking. On the most general level, masking can be regarded as the decreased visibility of an object (the target), through another object (the mask). However, the stimulus parameters used in this experiment - specifically the physical stimulus strength (intensity) of the inner patch specifying the mask in this scenario - are rather untypical for masking-experiments. Typically in metacontrast-masking, the target-stimulus is masked by a surrounding stimulus that is at least comparable in physical strength or mask energy (duration and intensity (Breitmeyer, 2006)). Here, a peri-liminal target-stimulus would surround a subliminal mask. However, in current models of masking, a neural activation pattern of a target that is dependent on physical stimulus strength, is masked through a disturbance in feedback-mechanisms (e.g. close temporal succession of a second stimulus) (Enns & Di Lollo, 2000; Kiefer, et al., 2011; Lamme & Roelfsema, 2000). In line with these theories, masking-experiments systematically varying mask-target energy ratio (M/T) show that the physical strength (intensity) of the mask in relation to the target is directly related to the metacontrast-masking effect (Alpern, 1953). Based on these studies, a masking-effect due to a subliminal mask that is surrounded by the target (further decreasing its physical strength in comparison to the target) would be expected to be small, if at all present. On the other hand, the effect size found in our study indicates a strong effect ($\eta_p^2 = .53$). However,

generally, it is difficult to compare the effects of masked high-intensity targets with those of the periliminal target-stimuli used in this study.

Furthermore, it is unlikely that subliminal stimuli - in spite of being imperceptible - could have acted as temporal or spatial attentional cues, such that target detection was affected by attention. If the subliminal stimulus train would have acted as spatial attentional cue, performance should have improved for targets presented at the cued location. On the other hand, compared to targets presented alone, performance for targets presented at the uncued location in the opposite hemifeld should have been impaired. Temporal cueing should also improve target detection regardless of the spatial locations of subliminal train and target. However, detection performance was impaired for targets presented at the same location as the subliminal stimulus train, and unchanged for targets presented at the opposite side, indicating that subliminal trains did not act as temporal or attentional cues.

Several other explanations centre on the selective sample used in the current experiment. First, the number of participants in both groups is comparatively small. For young participants (Figure 4A), all of the individual thresholds for target-stimuli indicate no effect of subliminal stimulation on the opposite side. The distribution of thresholds during subliminal stimulation on the same side however, indicates an increase for all but one participant. For the elderly (Figure 4B), again all of the individual thresholds for target-stimuli indicate no effect of subliminal stimulation on the opposite side. However, individual thresholds during subliminal stimulation on the same side indicate diminished inhibitory functioning in three out of five participants. This effect was not pronounced enough to lead to a significant interaction effect between the young and the elderly. On the other hand, using this set-up, it is not possible to exclude a tendency to diminished inhibitory response functioning. Future studies will require a larger sample size in order to clarify this issue.

Second, individuals that are likely to sign up for experimental work are also likely to engage with their environment more than other people their age. These participants are more likely to exhibit 'Brain maintenance' (Nyberg, Lovden, Riklund, Lindenberger, & Backman, 2012). Due to individual differences, some participants show little or no age-related declines in cognitive functions. Engagement in socially, mentally and physically stimulating activities in old age helps keeping the brain functioning and cognitively performing well. Thus, individuals tested in this experiment might 'maintain' their brain-activity better than other persons of the same age.

Third, it is feasible that the age of the participants tested in this study was not comparable to the age of the macaques. The age of senescent macaques was 26 to 30 years, an age equivalent to 90 years in humans (Leventhal, et al., 2003; Schmolesky, et al., 2000), while participants used in the current

experiment were between 65 to 75 years. The issue of the age of elderly participants was also raised by a study investigating spatial frequency selectivity in the elderly. According to physiological studies in senescent monkeys (Leventhal, et al., 2003; Schmolesky, et al., 2000; Zhang, et al., 2008), spatial frequency selectivity has been found to broaden with age. Contrary to the predictions from these studies, spatial frequency selectivity did not change in elderly humans (Govenlock, Taylor, Sekuler, & Bennett, 2010). The age-range of the elderly participants used in this study was 62 to 77 years, similar to the age-range used in the present study. As suggested by Govenlock, et al. (2010), the apparent discrepancy between physiological findings in senescent macaques and behavioural findings in elderly human could be due to a possible interaction between age and the effects of anaesthesia that was administered to the monkeys.

Furthermore, in a study investigating the effects of aging on orientation tuning, predicted changes in orientation tuning based on the same studies in senescent macaques (Leventhal, et al., 2000) could not be confirmed (Delahunt et al., 2008). Elderly participants did in fact show higher contrast and orientation discrimination thresholds (as predicted). Yet, the difference in discrimination thresholds vanished when contrast thresholds were taken into account. Similarly, differences in orientation tuning could not be found when plotted as multiples of contrast detection threshold. In the above-mentioned studies, losses in contrast sensitivity alone can account for the result of increased discrimination thresholds and broader tuning in the elderly.

In our study, we did take contrast-sensitivity into account as well. We estimated individual perception-thresholds in Phase I of the experiment. Similar to the studies mentioned above, we found the expected significant decrease in contrast-sensitivity for the target-stimulus for elderly as compared to young participants. Interestingly, the expected disadvantage of elderly participants due to the aging visual system seems to be attenuated by the presentation of a train of stimuli. Senescent cells in V1 (Schmolesky, et al., 2000) and V2 (Yu, et al., 2006) exhibit increased spontaneous activity resulting in decreased signal-to-noise ratios. That is, with decreased signal-to-noise ratios the benefit of repeated stimulation could be enhanced in the elderly as compared to younger participants. In sum, we suggest that stimuli escaping conscious perception together with near-threshold stimuli presented at the same location are subject to an inhibitory mechanism, which normally inhibits visual noise. This response seems not to be entirely restricted to a group of younger participants, but is - at least to some extent - evident in elderly with an age of up to 75 years.

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Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt,

dass ich die vorliegende Arbeit selbstständig und ohne unerlaubte Hilfe verfasst habe;

dass ich die Doktorarbeit an keiner anderen Universität eingereicht habe und keinen Doktorgrad in dem Promotionsfach Psychologie besitze;

und dass mir die zugrunde liegende Promotionsordnung der Mathematisch-Naturwissenschaftlichen Fakultät II (Name geändert zur Lebenswissenschaftlichen Fakultät) vom 17.01.2005, zuletzt geändert am 1.02.2006, veröffentlicht im Amtlichen Mitteilungsblatt der HU Nr. 34/2006, bekannt ist.

Berlin, den 20.7.2014

Isabelle Bareither